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Zebrafish Heart: A Model for Cardiovascular Research and Regeneration

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Zebrafish models are pivotal in cardiovascular research, offering a unique platform to study heart development, disease mechanisms, and potential therapies. Their transparent embryos and genetic manipulation tools, like CRISPR/Cas9, enable precise replication of human cardiac conditions such as heart failure, arrhythmias, and congenital defects. This accuracy is crucial for understanding disease pathways and testing new treatments effectively. These models simulate cardiac dysfunction through chemical disruptions, serving as robust platforms for drug discovery and testing. Their exceptional ability to regenerate heart tissue post-injury provides insights into treating human cardiovascular diseases. Various models, including surgical resection and genetic ablation,

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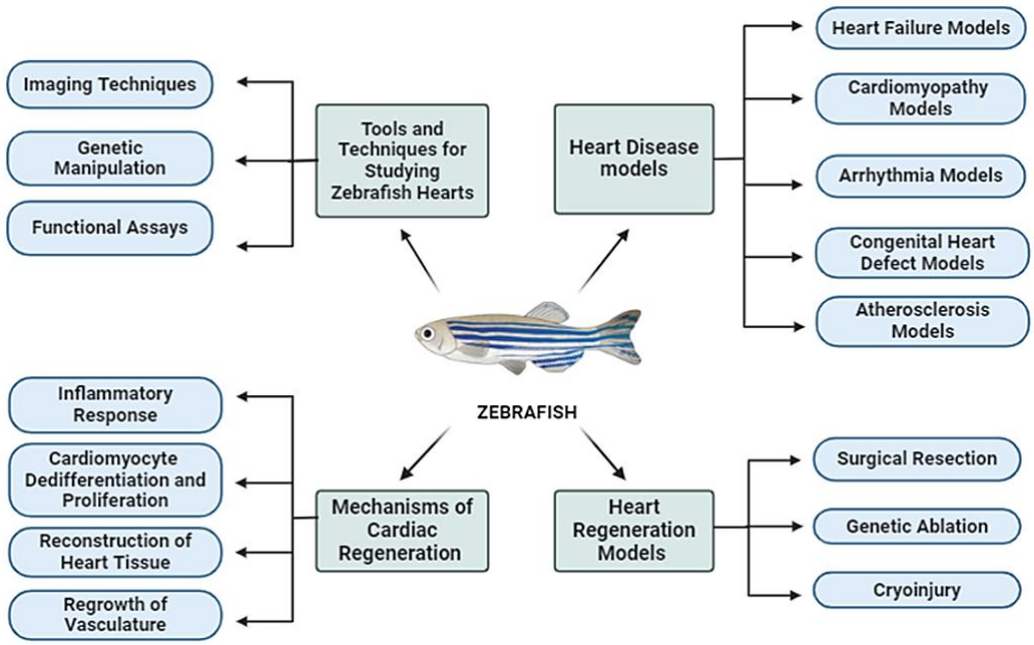
allow scientists to explore the mechanisms of cardiac regeneration, uncovering the roles of inflammation, cardiomyocyte dedifferentiation, and tissue reconstruction. Advanced imaging techniques like confocal microscopy offer detailed insights into cardiac dynamics, while electrophysiological recordings deepen the understanding of heart rhythms and abnormalities. High-throughput screening methods in zebrafish accelerate drug discovery efforts, aiding in the identification of potential treatments for diverse cardiovascular conditions. In essence, zebrafish models are invaluable tools for unraveling cardiovascular biology, clarifying disease pathways, and developing targeted therapies, with profound implications for improving cardiovascular disease management globally.

GRAPHICAL ABSTRACT



Keywords: Cardiovascular research; genetic manipulation; CRISPR/cas9; electrophysiological recordings; high-throughput screening; imaging techniques.

ABBREVIATIONS

CRISPR : Clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeat-associated
TALEN : Transcription activator-like effector nucleases
dHAND : Differentiation and hyperplasia-activated neural transcription factor
DCM : Dilated cardiomyopathy

1. INTRODUCTION

Cardiovascular diseases (CVD) constitute a group of illnesses that affect the heart and blood vessels of the body including coronary artery disease, hypertension, heart failure, stroke and so forth. The combined impact of these conditions on global morbidity and mortality constitutes a major public health concern. The World Health Organization (WHO) estimates that CVDs are the world's largest cause of death, accounting for 17.9 million fatalities per year [1].

CVDs could aggravate the healthcare system, national economies, as well as the overall society and individual well-being of all people. The intricate relationship between comorbidities, environmental variables, lifestyle decisions, and genetic predispositions adds to the complexity of cardiovascular conditions. Therefore, we are all in need of novel research strategies in order to illuminate the disease's mechanisms, define the disease targets, and obtain effective treatments.

Zebrafish, or *Danio rerio*, have been shown to be a promising model organism in this regard for the

research of cardiovascular disorders. Zebrafish, a freshwater fish, have a circulatory system that is very similar to that of humans. They maintain the same structural organization as human genes, which improves understanding of the interactions among genes and the pathways leading to human diseases. Also, the zebrafish's circulatory system resembles that of vertebrates, such as having two chambers in its heart and the same cardiac cell types, in addition to all the retained developmental mechanisms [2].

The word “zebrafish” actually comes from the characteristic stripes this fish has, which look like those of a zebra. Which normally ranges from 3 to 5 cm in body length, a slim body, and a clear zebra stripe pattern running along the side [3]. Zebrafish breed quite frequently. An individual female zebrafish can produce between 200 and 300 eggs during an individual spawning cycle, while some can produce even more [4]. The reproductive cycle is brief, with embryos growing externally inside a transparent chorion (egg membrane) [5]. In 2003, the zebrafish genome sequence was completed, showing that it was highly comparable to that of the human genome as regards the genes. Humans and zebrafish have more than 70% similar genes, many of which are involved in the immune system, its development, and its role in various disease processes [6]. The zebrafish might possess a great feature of genetic tractability, which makes this species an ideal model for genetic engineering research to be conducted. Targeted mutations can be induced in transgenic lines by introducing precision changes either into non-coding regions, exons, or introns of the zebrafish genome using methods such as CRISPR/Cas9 gene editing [7]. The zebrafish has a remarkable healing capacity, particularly in those tissues where regeneration occurs, such as the fins and the heart. They serve as an exemplary model not only in the research of heart regeneration but also in the mechanisms involved in repairing dysfunctional heart tissue within the first few days after the injury [8]. During cardiac injury, as a part of the regeneration cells, cardiomyocytes (heart muscle cells) at the site of the injury undergo a process of (i) dedifferentiation and (ii) proliferation. Due to this action, the damaged tissue is replaced by these cells, Which are capable of dividing to fulfill necessary functions. Research has indeed unveiled more than one pathway, including the FGF signaling system, the Wnt/β-catenin, and the Notch pathway, that significantly contributes to zebrafish heart regeneration [9,10]. The transparency of zebrafish embryos is among their beneficial characteristics for scientific study. Without the need for invasive treatments, this transparency enables researchers to view interior structures under a microscope, including heart development [11]. Indeed, advanced techniques, e.g., time-lapse photography and confocal microscopy, enable researchers to distinguish cellular functionality and organ *growth in vivo* [12,13].

In this article, we will explore how zebrafish models have contributed to our understanding of heart development and disease, highlighting their significance in advancing cardiovascular research and ultimately improving clinical outcomes for patients with cardiac disorders.

2. HEART DEVELOPMENT IN ZEBRAFISH

Zebrafish embryos hearts develop their hearts in an intricate and fascinating process that provides an understanding of both congenital cardiac abnormalities and normal cardiac development. Understanding these stages is useful not only in the research of cardiovascular disorders but also in the development of prospective treatments. The essential phases of the zebrafish heart's development are broken out in detail below:

2.1 Zygote Phase (0-0.75 days post-fertilization)

Heart development starts soon after fertilization. The zebrafish embryo rapidly divides its cells during its initial few hours without expanding its size. The zygote is divided into smaller cells known as blastomeres during these synchronous earlier divisions, which do not undergo growth [14].

2.2 Cleavage, Blastula, and Gastrula Phase (0-10 hours post-fertilization)

2.2.1 Cleavage

The blastula stage starts with the cleavage, which happens every 15 to 30 minutes and lasts until there are roughly 1,000 cells. Zebrafish cleavage is incomplete because it is meroblastic [15].

2.2.2 Blastula

At approximately 3.3 hpf, the embryo goes through a critical developmental stage called the Midblastula transition (MBT), during which zygotic gene transcription starts. Prior to MBT,

the embryo is primarily regulated by maternal mRNAs and proteins found in the egg. Following MBT, cells begin to interact and migrate more complexly, getting ready for gastrulation, which is when the main cell lineages start to become apparent [16].

2.2.3 Gastrulation (5.25 hpf onwards)

As the blastula stage develops, the cells on the surface of the blastoderm begin to shift toward the embryo's ultimate dorsal side, indicating the commencement of gastrulation. The definition of the embryonic axis and the establishment of the embryo's basic body plan rely on this movement [17].

2.3 Segmentation Phase (10-24 hours post-fertilization)

At this stage, the embryo starts developing somites, which are segmented body structures [18]. Bilateral cardiac crescents are formed when cardiac progenitor cells move toward the midline and are detected by the expression of certain markers, such as nkx2.5. The subsequent stage of heart tube creation depends on this alignment [19].

2.4 Pharyngula Pase (24-48 hours post-fertilization)

2.4.1 Heart tube formation (24-26 hours post-fertilization)

A primitive heart tube is formed when the bilateral cardiac crescents unite at the midline, 24–26 hours after fertilization. This tube starts as a straight line and differentiates into the primary heart muscle [20].

2.4.2 Looping (36 hours post-fertilization)

The heart tube will start a fundamental procedure, looping, which will then position the heart chambers spatially in the correct order. The heart begins to bend and fold into an S-shaped shape, defining the placement of the atria and ventricles. Another factor governing looping is likely to be genetic regulation of the genes such as dHAND and pitx2 [21,22].

2.5 Hatching Phase (48-72 hours post-fertilization)

The embryo starts to break out of the chorion that protects it. After 48 hours, the heart tube has already looped and the atria and ventricles are becoming more distinguishable from each other. Blood begins to pump from the heart, though slowly at first and irregularly, but it eventually stabilizes [23].

2.6 Larval Phase (72 hours post-fertilization onward)

2.6.1 Chamber maturation (3-7 days post-fertilization)

3–7 days after conception, the chamber reaches maturity. The heart will continue to mature during the next few days. The chambers keep expanding and becoming more functional as the myocardium thickens. After the formation of an atrioventricular canal, it is possible to establish the borders between the atrium and the ventricle [24].

2.6.2 Development of the conductive system (5-7 days post-fertilization)

The changes in the rate of heartbeat result from the occurrence of pacemaker cells which are responsible for the process. To maintain effective blood circulation throughout the developing body and synchronize the heartbeat, the conductive system needs to develop [25].

2.7 Juvenile and Adult Stages

The adult zebrafish has a remarkably organized and effective cardiac structure that was established during the development process. The adult zebrafish heart has a single-loop circulation system with clearly delineated atria and ventricles [26].

Following injury, the zebrafish's ability to replace missing or injured heart tissue becomes apparent. This process has been extensively investigated to gain knowledge about human cardiac repair and regenerative medicine. Zebrafish prove to be exceptional models for research into the cardiovascular area because they progress a whole development process and have the capacity as adults for regeneration.

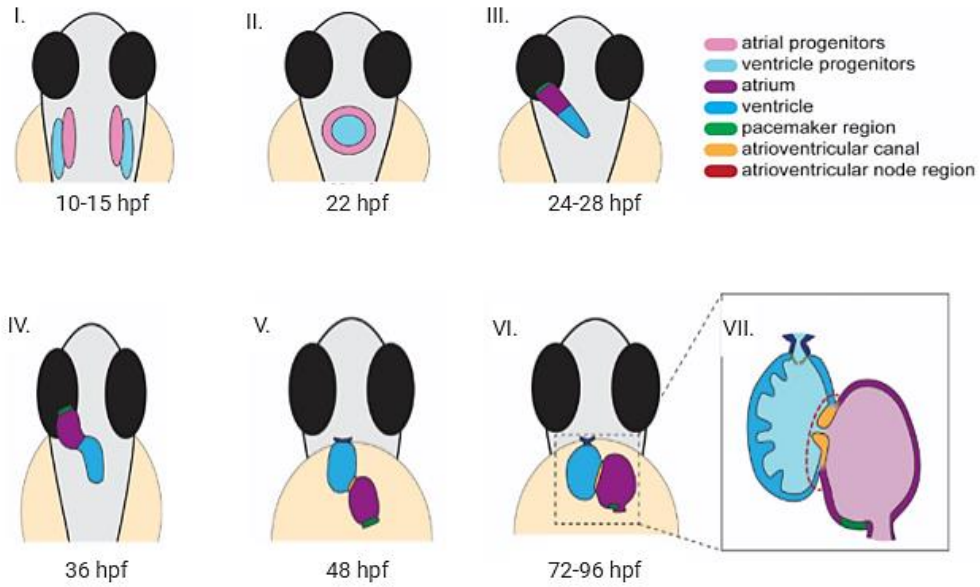


Fig. 1. Zebrafish heart development stages from 10 to 96 hours post-fertilization

i. Articular and ventricular cardiac progenitors are found in the anterior lateral plate mesoderm by 15 hpf.
ii. By 22 hpf, the cardiac disc appears as cardiac progenitors cover endocardial cells near the midline.
iii. The linear heart tube begins to develop and jog to the left at 24 hpf, preparing the heart for looping.
iv. The heart tube loops to the right at 36 hpf. The Atrioventricular (AV) canal (orange) starts to form between the heart chambers.
v. At 48 hpf, the heart chambers begin to inflate and extend outward. The bulbous arteriosus (dark blue) and the AV canal (orange) proceed to grow and mature.
vi. From 72 to 96 hpf, the heart chambers enlarge and align next to one other.
vii. The trabeculae, which are finger-like muscular projections on the inner wall of the ventricle, and the endocardial leaflets (orange) of the AV canal can be observed in this cross-section of a 96-hpf heart. (i-iv) Dorsal Views (v-vii)Ventral views

3. ZEBRAFISH MODELS OF HEART DISEASE

3.1 Heart Failure Models

Heart failure (HF) is a complex pathophysiological disease characterized by the heart's inability to adequately respond to systemic demands [27].

3.1.1 Genetic manipulation models

A zebrafish model was created to study the electrophysiological characteristics of ventricular hypertrophy and diastolic dysfunction caused by genetically knocking down the myosin-binding protein C gene (mybpc3). When mybpc3 was genetically knocked down in zebrafish using morpholino, the result was ventricular hypertrophy, increased myocardial wall thickness, and diastolic heart failure, which was manifested as decreased diastolic relaxation velocity, atrial dilatation, and pericardial effusion [28].

Under pathological circumstances, the import of mitochondrial precursor proteins is controlled by the translocases of the mitochondrial outer membrane (Tom) complex. This theory was examined by knocking down Tom70 with siRNA. There was a significant increase in cell size due to the lack of pro-hypertrophic stimulants. As basal levels of Tom70 expression were reduced, the alteration in the α-MHC, ANP, and BNP expressions was noted; however, the survival of the cells remained unchanged [29].

3.1.2 Chemical induction model

A procedure or protocol of isoproterenol chronicle stimulation, which eventually leads to

cardiac dysfunction, was modeled using both developing and maturing zebrafish. The research findings revealed that sustained, 14-day ISO stimulation resulted in severe heart failure in adult zebrafish, along with β1-AR downregulation, brain natriuretic peptide (BNP), and GRK2 (G protein-coupled receptor kinase 2) overexpression. The phase was characterized by marked inflammation and cell injury, although fibrosis was not evident visually [30].

An adult zebrafish model of cardiomyopathy caused by doxorubicin was developed. Two alternate methods of intraperitoneal (IP) injection—Classic IP injection and Alternative IP injection—were described, along with a comprehensive procedure for creating this DIC model in adult zebrafish [31].

3.2 Cardiomyopathy Models

Cardiomyopathy is an anatomical and pathologic diagnosis that refers to heart muscle or electrical malfunction. Cardiomyopathies are a variety of diseases that often eventually develop into irreversible heart failure with elevated mortality and morbidity rates [32].

3.2.1 Genetic mutations

A model of adult-onset autosomal recessive dilated cardiomyopathy caused by GATAD1-associated dilated cardiomyopathy in zebrafish was developed. By injecting a Tol2 plasmid encoding fluorescently-tagged GATAD1, it was found that GATAD1 protein localized in the nucleus and in the sarcomeric I-band of zebrafish embryos, along with cardiac expression of Gatad1 transcripts using whole-mount in situ hybridization. Fish lines with GATAD1 knockouts were created using TALEN technology, and a transgenic fish line was created to express the human DCM GATAD1-S102P (GATA zinc finger domain containing 1) mutation in cardiomyocytes [33].

GTPBP3(GTP Binding Protein 3) knock-out zebrafish were shown to have abnormal mitochondrial tRNA metabolism. These mitochondrial dysfunctions caused the abnormal development of the heart embryo and decreased the ventricular fractional shortening in zebrafish mutants. However, the GTPBP3 knock-out zebrafish showed a disorganized pattern of cardiac fibers in the ventricles and the hypertrophy of cardiomyocytes [34].

3.2.2 Pharmacological models

A model of dilated cardiomyopathy was generated using zebrafish larvae that were exposed to 5 to 10 μM terfenadine for a whole day. Terfenadine is a well-known cardiotoxic substance that causes dilatation of the ventricles. Blood was often collected and clotted in the chamber of zebrafish treated with terfenadine, and circulation was noticeably decreased. An appearance of edema-like fluid and swelling in both atria and ventricles were observed. There was a considerable increase in the ventricular area and a marked decrease in cardiac contractility [35].

Using the transparent zebrafish, two new pharmacological models, epinephrine-dependent heart failure and isoprenaline-dependent heart failure, have been proposed lately. Although the pigmentation in the body was reduced due to a sublethal dose of isoetharine at 7.50 mM, there was a significant decline in the heart rate (−28.70 beats/min) at the epinephrine dose of 0.5 mM that can be associated with its cardiotoxicity [36].

3.3 Arrhythmia Models

Cardiac arrhythmia is a condition that affects a heart muscle, leading to an irregular rhythm of the heartbeat. These abnormal cardiac excitation waves either initiate, propagate, or in combination with each other cause arrhythmias [37].

3.3.1 Ion channel mutations

The generation of a transgenic zebrafish model for arrhythmia conditions using the sodium channel of the human heart-type SCN5A-D1275N mutation was carried out. Cardiac diseases that are evident in human beings, ranging from sinus node dysfunction to conduction diseases, ventricular and atrial arrhythmias, and dilated cardiomyopathy, have been associated with this particular mutation. The transgenic zebrafish with the SCN5A-D1275N mutation displayed anomalies related to the conduction system, bradycardia, and early mortality. Both conventional ECG and video microscopy approaches were utilized for a thorough study of the cardiac morphology and phenotypes [38].

The assessment of the employing of the zebrafish heart arrhythmia model was done to mark out the clinical implications of the carriers of the KCNQ1 (Potassium Voltage-Gated Channel

Subfamily Q Member 1) mutations. Therefore, to configure homozygous knockout kcnq1 zebra fish (del/del), the CRISPR/Cas9 was applied and the human Kv7.1/MinKchments were expressed in kcnq1del/del embryos, which was further necessary to explore LQTS (Long QT Syndrome) mutation pathogenicity [39].

3.3.2 Drug-induced arrhythmias

Seven medications that are known to be cardiotoxic to humans were evaluated for their cardiovascular toxicity: A trial was initiated to explore the efficacy of aspirin, cyclophosphamide, terfenadine, nimodipine, clomipramine hydrochloride, quinidine, and verapamil hydrochloride. The outcomes revealed that aspirin exhibited tachycardia in zebrafish, while clomipramine hydrochloride, nimodipine, quinidine, terfenadine, cyclophosphamide, and verapamil hydrochloride induced bradycardia. Both terfenadine and quinidine produced AV blocks. The Zebrafish was given imidapine and was in atrial arrest with a regular but slow ventricular heart rate [40].

The change in the rate of heartbeat (HBR) in zebrafish, hERG (human ether-à-go-go related gene) fluorescence polarization (hERG-FP), and ionic current change using a patch clamp (hERG-PC) were used to assess the bradycardia of the following compounds: propranolol, chlorpromazine, demeclocycline, astemizole, droperidol, E-4031, fluconazole, terfenadine, disopyramide, fluoxetine, pimozide, haloperidol, propafenone, sotalol, and thioridazine. The IC50 value could be used from the hERG-FP or hERG-PC assay, where the % of HBR is equal to log(IC50, hERG-FP)×19.5 or log(IC50, hERG-FP)×19.6, respectively, designed to investigate the compounds tested. Additionally, the model for predicting Drug-induced bradycardia was developed based on *in vivo* and *in vitro* experiments intended for high-speed toxicological screenings [41].

3.4 Congenital Heart Defect Models

Congenital heart disease refers to cardiac structural defects that develop prior to birth. During pregnancy, these problems develop in the growing fetus inside the uterus [42].

3.4.1 Gene editing

The Pbx gene variant likely had an effect of genetic modification, which was observed in the development of the zebrafish's heart by transgenic genomic (CRISPR-Cas9) genome editing. The human PBX3 p.A136V variation was carefully inserted into the homologous zebrafish pbx4 gene (pbx4 p.A131V) using a single-stranded PCR. The PBX3 p.A136V variation may act as a CHD modifier allele. Nevertheless, the deletion of the recognized cardiac specification factor, Hand2, resulted in an enhancement of the embryonic cardiac morphogenesis phenotype by the pbx4 p.A131V variation [43].

A zebrafish strain with a Dihydrofolate reductase gene knock-in (DPHR KI) was created to help with the early investigation of the mechanisms underlying congenital heart disease. Microscopic analysis of KI (homozygous) specimens at 6 days post fertilization (dpf) revealed coiled tails, heart compressions, and pericardial effusions. The cut slides of zebrafish on KI-homozygous with hematoxylin and eosin staining showed morphologic defects, including reduced atrium and ventricle, compared with wild-type zebrafish larvae. In brief, the DHFR (dihydrofolate reductase) protein level increased significantly both in DPHR KI (Differential Pulmonary Hypertension Re-emersion) zebrafish heterozygotes and homozygotes, according to the western blot examination [44].

3.4.2 Chemical interference

The decrease of ventricular cardiomyocytes in hdac1 mutants was shown to be largely caused by the ectopic production of the Retinoic acid-responsive gene (ripply3), which functions as a transcriptional corepressor for Tbx1. It was shown that the epigenetic regulator Hdac1 mediates transcriptional repression, which in turn promotes outflow tract (OFT) development by directly inhibiting the expression of the Retinoic acid-responsive gene ripply3 in second heart field (SHF) progenitors [45].

Vascular endothelial growth factor-induced intersegmental vessel (ISV) growth arrest can be avoided by overexpressing Dlc1. The discovery of Vegfr2 pathway suppression implies that Dlc1 functions *in vivo* directly downstream of the Kdrl axis. In a zebrafish embryo, Dlc1 is a key regulator of cardiovascular development by signaling Vegfa/Kdrl/Nrp1 [46].

3.5 Atherosclerosis Models

The most prevalent kind of cardiovascular disease (CVD), known as atherosclerosis or coronary artery disease (CAD), is primarily caused by the accumulation of cholesterol and

inflammation of the major arteries. Atherosclerosis may eventually result in myocardial infarction (MI) and stroke, two of the condition's clinical consequences [47].

3.5.1 Dietary manipulation

Apolipoprotein A-II's function in ezetimibe-induced atherosclerosis produced by high-cholesterol diets was assessed. Morphininos that target the zebrafish Apoa2 mRNA were employed to knock down Apo AII to examine the function of Apo A-II in the anti-atherosclerotic action of ezetimibe. SiRNA transfection of HNF4c (Hepatocyte nuclear factor), PPARα ((peroxisome proliferator-activated receptor alpha), and SREBP1(Sterol Regulatory Element-Binding Protein 1) siRNA into HepG2 (Hepatocellular Carcinoma G2) cells was achieved to verify if ezetimibe is regulatory or activational inducer of Apo A-II expression. Ultimately, it was shown that ezetimibe enhanced Apo A-II expression through the transcription factors PPARα and HNF4 [48].

The defensive properties of Dendrobium huoshanense C. Z. Tang et S. J. Cheng polysaccharide against high-cholesterol diet-induced atherosclerosis in zebrafish were investigated. The primary outcomes of the study indicate that DHP significantly improved the levels of total cholesterol (TC), triglycerides (TG), malondialdehyde (MDA), reactive oxygen species (ROS), and plaque formation. It also significantly reduced neutrophil recruitment and increased superoxide dismutase (SOD) activity. These improvements were observed in the context of HCD-induced lipid deposition, oxidative stress, and the inflammatory response [49].

3.5.2 Genetic modifications

An Apoc2 mutant zebrafish with lost Apoc2 function was created in order to provide a genetic model of hypertriglyceridemia. Apoc2 mutants exhibit a phenotype that is similar to that of APOC2-deficient human patients, including decreased plasma lipase activity, chylomicronemia, and severe hyper-triglyceridemia [50].

Using a zebrafish (*Danio rerio*) model system, the causal genes (abcg5, abcg8, col4a3bpa, col4a3bpb, myrf, st3gal3, ywhaqa, and ywhaqb) for LDL levels of cholesterol along with their downstream consequences for atherosclerosis were described. The CRISPR/Cas9 approach was utilized to target the genes using fluorescently tagged macrophages (Tg [mpeg1:mCherry]) and neutrophils (Tg [mpo:EGFP]). The larvae with mutations in the genes abcg5, abcg8, col4a3bpb, and ywhaqb appeared to be more resistant to the atherosclerotic phenotype, based on the decreased lipid buildup and the decreased co-localization of neutrophils and macrophages in the vasculature [51].

4. MODELS OF HEART INJURY IN ZEBRAFISH

Cardiac regeneration in zebrafish is a prominent area of research because, unlike humans, zebrafish have a remarkable ability to regenerate their hearts after injury. Understanding this capability can provide crucial insights into potential treatments for heart disease in humans, who typically respond to cardiac injury with scar formation and limited regeneration, leading to diminished function and heart failure. Here's a closer look at how cardiac regeneration in zebrafish is studied, the mechanisms behind it, and its potential implications for human medicine.

To study cardiac regeneration, a model of heart injury in zebrafish has to be created. This is commonly achieved through:

4.1 Surgical Resection

Apex resection (AR) is a widely used zebrafish model for researching heart regeneration, which involves excising 10–20% of the heart in the apex area and then monitoring the regeneration process until the heart regenerates completely in 60 days. Assessments of zebrafish heart regeneration can be conducted in two ways: in three dimensions (using CUBIC Tissue Clearing and 2-Photon Imaging to analyze entire, tissue-cleared hearts) and in two dimensions (using immunohistochemistry of heart sections) [52].

Zebrafish hypoxia induction during ventricular amputation may be involved in controlling the regeneration of the damaged heart tissue. Hypoxia was induced using phenylhydrazine, hypoxyprobe, and Cre/Tamoxifen therapy. Hypoxia stimulates cardiomyocytes to dedifferentiate and proliferate during heart regeneration in zebrafish, as discovered using a combination of O2 disruption, conditional transgenics, *in vitro* cell culture, and microarray analysis [53].

4.2 Genetic Ablation

Zebrafish lines can be genetically modified to express a toxin under a cardiomyocyte-specific

promoter, specifically destroying heart cells when activated.

Numerous epicardial lineage cells isolated from zebrafish bearing a transgenic reporter for the pan-epicardial gene tcf21 were subjected to transcriptome profiling. The function of caveolin 1 (cav1), a scaffolding protein and key component of caveolae that is found in each epicardial subgroup, was investigated. The genome-edited cav1 mutant zebrafish exhibited adult cardiac morphology and heart development that was essentially normal, but it also revealed significant abnormalities in injury-induced cardiomyocyte proliferation and regeneration of the heart [54].

In situ hybridization and transgenic reporter assays yielded data showing that the damage-induced expression of both fibronectin paralogues, fn1, and fn1b, takes place in epicardial cells, while the local upregulation of itgb3 disproportionately happens in the cardiomyocytes neighboring the injury site. The next day after damage, fn1 is up-regulated at a higher degree than the other paralog in this chamber, and epicardial production of fn1 shifts to the site of injury [55].

4.3 Cryoinjury

The cardiac tissue at the site of operation suffers a localized freeze-thaw injury that gives it a resemblance to the damage and scarring observed in human heart attack as compared to resection of tissue due to surgical cutting [56].

By employing the method of cryoinjury, the zebrafish heart-infarct model was created, and data about the chemical expression of Tenascin-C was shown. The degree of vimentin-positive fibroblasts is pertinent to the remodeling of myocardium beyond the margin of myocardial infarction. It became evident in the TUNEL assay that cardiomyocyte cell replication was up-regulated in regenerating after infarction and remained elevated in the post-infarct period. Analysis of the ECG showed that the restoration of heart muscle function is a result of the cardiac muscle's reconstruction. Analysis of the ECG showed that the restoration of heart muscle function is a result of the cardiac muscle's reconstruction [57].

A technique for cryoinjuring zebrafish ventricles to produce a repeatable disc-shaped infarct was demonstrated. Acid-Fuchsin Orange-G (AFOG) staining, which distinguishes between cardiac and fibrotic tissues, was used in histological research to assess the degree of heart regeneration as compared to scarring. Immunofluorescence experiments were conducted with specific antibodies to detect the location of these marker proteins [58].

5. MECHANISMS OF CARDIAC REGENERATION

5.1 Inflammatory Response

The significance of inflammation in increasing cell cycle activity was investigated in the context of cardiac preconditioning and regeneration. Two models of cardiac preconditioning and cardiac infarction in adult zebrafish were employed: a thoracotomy and a cryoinjury. Following thoracotomy, leucocytes, and cycling heart cells were first spatiotemporally characterized. The results of this research implied influence of the mitotic activity on the infiltration of the inflammatory cells [59].

In zebrafish, estrogen stimulates the inflammatory response, thus intensifying the regenerative process and making the heart regeneration faster. According to transcriptome analysis, regeneration of the zebrafish heart is rapid in females and can be increased by estrogen and the expression of estrogen receptors, particularly esr2a. Tamoxifen, an estrogen antagonist, can also decrease this process [60].

5.2 Cardiomyocyte Dedifferentiation and Proliferation

By inducing endocardial expansion and cardiomyocyte dedifferentiation, Tbx20 induction improved the regeneration of the zebrafish heart. The promotion of injury-induced cardiomyocyte proliferation via CM dedifferentiation, which results in the loss of CM cellular connections and the reexpression of cardiac embryonic or fetal gene programs, is achieved by specifically inducing Tbx20 in the adult myocardium [61].

Coordination of Wnt signaling inhibitors, such as Dickkopf 1 (Dkk1), secreted Frizzled-related protein 1 (sFrp1), Dkk3, and sFrp2, and Pak2/pS675-β-catenin signaling, promotes zebrafish heart regeneration by promoting CM dedifferentiation and proliferation [62].

5.3 Reconstruction of Heart Tissue

There was an absence of muscle tissue regeneration during this stage of redevelopment. Clones of the primordial muscle layer were

detectable in the regeneration by 30 days after injury, and by 60 days after trauma, a complete primordial layer had been restored, creating a single layer of cells on the border between trabecular and regenerated cortical muscle. This took place as the damaged muscular wall was regenerated by forming a new cortical muscle sheath [63].

Newly produced cardiomyocytes, endocardial cells, vascular endothelial cells, epicardial cells, and local cells such as primordial germ cells migrate, develop and integrate into the damaged tissue [64]. The endocardium forms the lining of the inner surface of the heart and is responsible for restoring the structural function of the heart and the epicardium surrounds the walls of the heart [65].

5.4 Regrowth of Vasculature

As soon as 15 hours after the damage, the affected area starts developing angiogenesis. Using vegfaa mutants that were brought to maturity by vegfaa mRNA injections at the one-cell stage, the function of vegfaa in heart regeneration was examined. Remarkably, vegfaa mutants undergo revascularization and coronary development upon damage. Effective revascularization of the damaged area is made possible by endothelial invasion, and this is required to promote the production of new tissue and accomplish effective heart regeneration [66].

6. MOLECULAR PATHWAYS

6.1 Transcription Factors

The DNA-binding domains of TBX5 (T-Box Transcription Factor 5) and MEF2C (Myocyte Enhancer Factor 2C) physically interact to activate the cardiac myosin heavy chain (MYH6) synergistically. Morpholino-mediated zebrafish tbx5 and Mef2c knockdowns indicate that these proteins' genetic connection is necessary for MYH6 (Myosin Heavy Chain 6) production as well as the survival and development of the heart in its early stages [67].

GATA4/5/6 specification at the cell autonomously operates upstream from tbx1 for a crucial function in strengthening the cardiac and inhibiting the pharyngeal mesoderm identity by analysis modeling performed on zebrafish with a single homolog for GATA4/5/6. The majority of lineages originating from mesendoderms exhibited dynamic gata5/6 expression [68].

6.2 Growth Factors

A small percentage of the cardiomyocytes in the zebrafish heart exhibit FGF(Fibroblast Growth Factor) signaling before injury, and the number of cardiomyocytes expressing this signal rises following amputation-induced damage. Cardiomyocyte death without injury was elevated, and the proportion of phosphorylated AKT (serine/threonine kinase) -positive cardiomyocytes was decreased when FGF signaling was inhibited [69].

In the case of zebrafish heart regeneration, Igf1r turns on the downstream signaling pathways that allow the cells to proliferate, differentiate, and anti-apoptotic pathways. Following cardiac damage, fewer proliferating cardiomyocytes were observed when dominant-negative igf1ra (dn-igf1ra) was expressed ectopically or when NVP-AEW541, a specific Igf1r inhibitor, was administered [70].

6.3 Cell Communication

Damage to the heart of adult zebrafish causes a substantial increase in the production of neuregulin1 (Nrg1) in perivascular cells. Myocardial Nrg1 overexpression promotes the proliferation of cardiomyocytes in response to injury, while inhibition of Erbb2, a Nrg1 co-receptor, inhibits this proliferation. Reactivating Nrg1 expression produces overt muscle hyperplasia, cardiomyocyte dedifferentiation, enhanced vascularization, and epicardial activation in uninjured zebrafish. It also causes cardiomegaly by persistently adding wall myocardium [71].

7. TOOLS AND TECHNIQUES FOR STUDYING ZEBRAFISH HEARTS

A variety of tools and techniques are available for studying zebrafish hearts at both structural and functional levels. Studying zebrafish hearts involves a nuanced approach that combines sophisticated imaging technologies, genetic manipulation, and functional assays to probe the underlying mechanisms of cardiac biology and disease.

7.1 Imaging Techniques

7.1.1 Confocal microscopy

Zebrafish hearts can be imaged in high resolution by using a confocal microscope, which

is a powerful imaging methodology. It works by employing an optical sectioning system that scans across the specimen with a laser, thereby removing out-of-focus light and allowing visibly clear, 3D-shaped views of the heart architecture [72].

Multiple approaches were adjusted to inject marker particles of fluorescent light into the vascular system of zebrafish and to visualize the movement in the walls of zebrafish hearts at microscopic resolution, with the potential for creating a list of blood flow capabilities at high resolution. Such information may help in explaining a broad range of references selecting structures into flow. The ability to record 2D cardiac wall motions of zebrafish with temporal and spatial resolutions adequate to describe the extremely dynamic intravital flow-structure environment has been made possible by recent developments in high-speed confocal imaging [73].

Non-destructive measurement of cardiac function can be achieved through the recording of cardiovascular parameters of zebrafish embryos and larvae with a laser-scanning confocal microscope. Blood vessel cell flow and velocity were shown in line-scan images created using laser-scanning microscopes. The use of tricaine was associated with the development of cardiac toxicity, which was also examined using the rapid scanning loop (laser scanning velocimetry) that studies the morphology and function of the developing myocardium [74].

7.1.2 High-speed video microscopy

In order to analyze the rapid and fast beating zebrafish heart, especially in embryos and larvae, this technique entails collecting a rapid series of videos at high rates of frame rate [75].

High-speed video microscopy can be used to examine blood circulation, hemodynamics, and heart function under a microscope (*Danio rerio*). A stereo microscope fitted with a high-speed camera captured a 10-second high-speed video of a zebrafish's beating heart and blood moving through its major capillaries while the fish were less than five days post-fertilization (dpf). MicroZebraLab and ImageJ software were used to analyze the videos and assess heart function [76].

7.1.3 Light sheet microscopy

By involving a thin layer of light collinear to the axis of detection, light sheet microscopy (usually called selective plane illumination microscopy, SPIM) illuminates the substance being examined. This strategy particularly enhances the resistance of the specimen to photodamage and photobleaching, an important advantage over long-term live imaging since the exposure of light to the specimen outside the focal plane is drastically reduced [77].

The design of a multi-planar light sheet fluorescence microscopy system and a programmable multi-planar illumination with a dielectric isosceles triangular array were produced. The *in vivo* zebrafish hearts imaging was subjected to assessment, fluorescent microspheres volumetric scanning was also tested and the three-dimensional illumination beam measurement was also evaluated [78].

7.2 Genetic Manipulation Tools

7.2.1 CRISPR/Cas9

The accurate editing abilities of (clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeat-associated 9) allow for the modification or knockout of particular genes. This approach is especially important in the development of various kinds of zebrafish with mutations identical to the ones discovered in human heart conditions, e.g., cardiomyopathies [79].

The cmlc2 promoter promotes Cas9 expression to knock down the nrp1 gene in heart muscle cells in a heat-shock-inducible manner using a novel CRISPR-based vector system for tissue-specific gene silencing. By employing this method, the function of neuropilin isoforms nrp1a and nrp1b in adult zebrafish hearts was examined in relation to cardiac injury and regeneration [80].

A phylogenetic study and the literature have found three zfactc genes: zfactc1a, zfactc1c, and cardiofunk (zfacta1b). CRISPR-Cas9 was utilized to characterize the editing of zfactc genes in zebrafish embryos, thereby validating these genes as cardiac-specific. The zfactc genes turned out to be crucial in heart development and as a hope for solving the upcoming relevance of humans to ACTC in the future [81].

7.2.2 Morpholino oligonucleotides

Morpholino Oligonucleotides (MOs) according to their mechanism are employed widely in

zebrafish cardiac models to prevent the translation of specific mRNA or changing pre-mRNA splicing as a means to study cardiac gene function. Thus, researchers could investigate the consequences of gene synthetic knockdown on cardiac development and pathology by our approach [82].

In order to disrupt the function of hand2, translation-blocking Morpholino Antisense Oligonucleotides targeting hand2 are designed and microinjected into the yolk of zebrafish embryos at the 1-4 cell stage. These "morphants" are then saved by co-injecting mRNA that codes for the matching cDNA. The effectiveness of the morpholino microinjections is then evaluated by phenotypic analysis in heart development when morpholino has been confirmed to be present in the yolk (co-injected with phenol red) [83].

7.3 Functional Assays

7.3.1 Electrophysiological recording

Electrocardiography (ECG) is a novel method for evaluating cardiac rhythm and detecting arrhythmias that was adapted from human cardiology. This may help understand the electrical behavior of a zebra heart which can be altered due to drug effects or genetic abnormalities [84].

A system for optical mapping of zebrafish hearts was built, utilizing two industrial-grade CCD (Charge-Coupled Device) cameras, the voltage-sensitive dye RH 237, and the calcium indicator dye Rhod-2. The rate dependence of voltage and calcium dynamics inside the atrial and ventricular chambers can be simultaneously observed by using affordable cameras and a shared 532-nm diode laser for excitation [85].

7.3.2 Pharmacological testing

The effects of multiple drugs on heart function can be evaluated simultaneously through high-throughput screening techniques. This has the potential to speed the development of novel medications for heart disease treatment [86].

Zebrafish (*Danio rerio*) embryos in a microtiter plate are used in an effective automated high-throughput screening technique that uses automated label-free heart rate determination. The graphical user interface of HeartBeat software allows for the automated measurement of heart rate and rhythm through the use of automatically collected bright-field data. The assay's sensitivity was illustrated by tracking heart rates throughout the developmental process of the embryo. More than 500 embryos can be processed throughout every 96-well plate due to the ability to score several embryos per well [87].

8. CONCLUSION

In conclusion, the use of zebrafish models in cardiovascular research has proven invaluable in unraveling the complexities of heart development, disease mechanisms, and regenerative processes. Through genetic manipulation, advanced imaging techniques, and functional assays, researchers have gained profound insights into the genetic and physiological parallels between zebrafish and human hearts. These models have enabled the study of various cardiovascular diseases, including heart failure, cardiomyopathies, arrhythmias, congenital heart defects, and atherosclerosis, offering platforms for drug testing and therapeutic development.

Moreover, the regenerative capabilities of zebrafish hearts have opened new avenues for understanding cardiac repair mechanisms. Studies on cardiac regeneration in zebrafish, particularly through models of heart injury such as surgical resection, genetic ablation, and cryoinjury, have shed light on the molecular pathways and cellular processes involved in tissue regeneration. These insights hold promise for potential therapeutic strategies targeting heart regeneration in humans, offering hope for more effective treatments for cardiovascular diseases.

Overall, zebrafish models have emerged as powerful tools in cardiovascular research, bridging the gap between basic science and clinical applications. Continued advancements in zebrafish research methodologies and technologies will likely lead to further discoveries and innovations in understanding heart health, disease prevention, and treatment strategies.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Amran MS, Bahar NB, Akash S. Perspective chapter: Physiology and pathology of the cardiovascular system. Novel Pathogenesis and Treatments for Cardiovascular Disease: IntechOpen; 2022.
2. Bally-Cuif L, Vernier P. Organization and physiology of the zebrafish nervous system. Fish Physiology. Elsevier. 2010; 29:25-80.
3. Driever W, Fishman MC. The zebrafish: Heritable disorders in transparent embryos. The Journal of clinical investigation. 1996;97(8):1788-94.
4. Hoo JY, Kumari Y, Shaikh MF, Hue SM, Goh BH. Zebrafish: A versatile animal model for fertility research. BioMed Research International. 2016;2016.
5. Hajam YA, Rani R, Sharma P, Kumar R, Verma SK. Zebrafish (*Danio rerio*): A versatile model for reproductive biology. Recent updates in molecular Endocrinology and Reproductive Physiology of Fish: An Imperative step in Aquaculture. 2021;105-20.
6. Patton EE, Zon LI, Langenau DM. Zebrafish disease models in drug discovery: From preclinical modelling to clinical trials. Nature Reviews Drug Discovery. 2021;20(8):611-28.
7. Sassen WA, Köster RW. A molecular toolbox for genetic manipulation of zebrafish. Advances in Genomics and Genetics. 2015;151-63.
8. González-Rosa JM, Burns CE, Burns CG. Zebrafish heart regeneration: 15 years of discoveries. Regeneration. 2017;4(3):105-23.
9. Gao J, Fan L, Zhao L, Su Y. The interaction of Notch and Wnt signaling pathways in vertebrate regeneration. Cell Regeneration. 2021;10:1-17.
10. Tang D, He Y, Li W, Li H. Wnt/β-catenin interacts with the FGF pathway to promote proliferation and regenerative cell proliferation in the zebrafish lateral line neuromast. Experimental & Molecular Medicine. 2019;51(5):1-16.
11. Teame T, Zhang Z, Ran C, Zhang H, Yang Y, Ding Q, et al. The use of zebrafish (*Danio rerio*) as biomedical models. Animal Frontiers. 2019;9(3):68-77.
12. Kamei M, Weinstein BM. Long-term time-lapse fluorescence imaging of developing zebrafish. Zebrafish. 2005;2(2):113-23.
13. Høgset H, Horgan CC, Armstrong JP, Bergholt MS, Torraca V, Chen Q, et al. *In vivo* biomolecular imaging of zebrafish embryos using confocal Raman spectroscopy. Nature Communications. 2020;11(1):6172.
14. Kane DA. Cell cycles and development in the embryonic zebrafish. Methods in Cell Biology. 1998;59:11-26.
15. Pathak NH, Barresi MJ. Zebrafish as a model to understand vertebrate development. The Zebrafish in Biomedical Research: Elsevier. 2020;559-91.
16. Kane DA, Kimmel CB. The zebrafish midblastula transition. Development. 1993; 119(2):447-56.
17. Yuen YF. Characterisation of calcium signalling in the external yolk syncytial layer during late blastula/early gastrula periods of zebrafish development: Hong Kong University of Science and Technology (Hong Kong); 2021.
18. Stackley KD, Beeson CC, Rahn JJ, Chan SS. Bioenergetic profiling of zebrafish embryonic development. Plos One. 2011; 6(9):e25652.
19. Wanga JO. Expression of NK2 homologs and cardiomyocyte differentiation during zebrafish development: Analysis of 8-26 somite stages: Howard University; 2002.
20. Stainier DY, Lee RK, Fishman MC. Cardiovascular development in the zebrafish I. Myocardial fate map and heart tube formation. Development. 1993;119(1): 31-40.
21. Angelo S, Lohr J, Lee KH, Ticho BS, Breitbart RE, Hill S, et al. Conservation of sequence and expression of Xenopus and zebrafish dHAND during cardiac, branchial arch and lateral mesoderm development. Mechanisms of Development. 2000;95(1-2):231-7.
22. Chin AJ, Tsang M, Weinberg ES. Heart and gut chiralities are controlled independently from initial heart position in the developing zebrafish. Developmental Biology. 2000;227(2):403-21.
23. Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. Developmental Dynamics. 1995;203(3): 253-310.
24. Singleman C, Holtzman NG. Analysis of postembryonic heart development and maturation in the zebrafish, *Danio rerio*. Developmental Dynamics. 2012;241(12): 1993-2004.

25. Sedmera D, Reckova M, DeAlmeida A, Sedmerova M, Biermann M, Volejnik J, et al. Functional and morphological evidence for a ventricular conduction system in zebrafish and Xenopus hearts. American Journal of Physiology-Heart and Circulatory Physiology. 2003;284(4): H1152-H60.
26. Singleman C, Holtzman NG. Growth and maturation in the zebrafish, *Danio rerio*: A staging tool for teaching and research. Zebrafish. 2014;11(4):396-406.
27. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey Jr DE, Drazner MH, et al. 2013 ACCF/AHA guideline for the management of heart failure: executive summary: A report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. Circulation. 2013;128(16):1810-52.
28. Chen YH, Pai CW, Huang SW, Chang SN, Lin LY, Chiang FT, et al. Inactivation of myosin binding protein c homolog in zebrafish as a model for human cardiac hypertrophy and diastolic dysfunction. Journal of the American Heart Association. 2013;2(5):e000231.
29. Li J, Qi M, Li C, Shi D, Zhang D, Xie D, et al. Tom70 serves as a molecular switch to determine pathological cardiac hypertrophy. Cell Research. 2014;24(8): 977-93.
30. Kossack M, Hein S, Juergensen L, Siragusa M, Benz A, Katus HA, et al. Induction of cardiac dysfunction in developing and adult zebrafish by chronic isoproterenol stimulation. Journal of Molecular and Cellular Cardiology. 2017; 108:95-105.
31. Ma X, Ding Y, Wang Y, Xu X. A doxorubicin-induced cardiomyopathy model in adult zebrafish. JoVE (Journal of Visualized Experiments). 2018(136): e57567.
32. Wexler R, Elton T, Pleister A, Feldman D. Cardiomyopathy: An overview. American Family Physician. 2009;79(9):778.
33. Yang J, Shah S, Olson TM, Xu X. Modeling GATAD1-associated dilated cardiomyopathy in adult zebrafish. Journal of Cardiovascular Development and Disease. 2016;3(1):6.
34. Chen D, Zhang Z, Chen C, Yao S, Yang Q, Li F, et al. Deletion of Gtpbp3 in zebrafish revealed the hypertrophic cardiomyopathy manifested by aberrant mitochondrial tRNA metabolism. Nucleic Acids Research. 2019;47(10):5341-55.
35. Gu G, Na Y, Chung H, Seok SH, Lee H-Y. Zebrafish larvae model of dilated cardiomyopathy induced by terfenadine. Korean Circulation Journal. 2017;47(6): 960.
36. Maciag M, Wnorowski A, Bednarz K, Plazinska A. Evaluation of β-adrenergic ligands for development of pharmacological heart failure and transparency models in zebrafish. Toxicology and Applied Pharmacology. 2022;434:115812.
37. Fenton FH, Cherry EM, Glass L. Cardiac arrhythmia. Scholarpedia. 2008;3(7):1665.
38. Huttner IG, Trivedi G, Jacoby A, Mann SA, Vandenberg JI, Fatkin D. A transgenic zebrafish model of a human cardiac sodium channel mutation exhibits bradycardia, conduction -system abnormalities and early death. Journal of Molecular and Cellular Cardiology. 2013;61:123-32.
39. Cui S, Hayashi K, Kobayashi I, Hosomichi K, Nomura A, Teramoto R, et al. The utility of zebrafish cardiac arrhythmia model to predict the pathogenicity of KCNQ1 variants. Journal of Molecular and Cellular Cardiology. 2023;177:50-61.
40. Zhu JJ, Xu YQ, He JH, Yu HP, Huang CJ, Gao JM, et al. Human cardiotoxic drugs delivered by soaking and microinjection induce cardiovascular toxicity in zebrafish. Journal of Applied Toxicology. 2014; 34(2):139-48.
41. Park MJ, Lee KR, Shin DS, Chun HS, Kim CH, Ahn SH, Bae MA. Predicted drug-induced bradycardia related cardio toxicity using a zebrafish *In vivo* model is highly correlated with results from *In vitro* tests. Toxicology Letters. 2013;216(1):9-15.
42. Sun R, Liu M, Lu L, Zheng Y, Zhang P. Congenital heart disease: Causes, diagnosis, symptoms, and treatments. Cell Biochemistry and Biophysics. 2015;72: 857-60.
43. Farr III GH, Imani K, Pouv D, Maves L. Functional testing of a human PBX3 variant in zebrafish reveals a potential modifier role in congenital heart defects. Disease Models & Mechanisms. 2018; 11(10):dmm035972.
44. Gong K, Xie T, Yang Y, Luo Y, Deng Y, Chen K, et al. Establishment of a dihydrofolate reductase gene knock-in zebrafish strain to aid preliminary analysis of congenital heart disease mechanisms.

Frontiers in Cardiovascular Medicine. 2021;8:763851.
45. Song Y. Epigenetic repression of retinoic acid responsive genes for cardiac outflow tract formation: University of Cincinnati; 2019.
46. Linnerz T, Bertrand JY. Dlc1 controls cardio-vascular development downstream of Vegfa/Kdrl/Nrp1 signaling in the zebrafish embryo. Biorxiv. 2021;2021(02): 11.430763.
47. Björkegren JL, Lusis AJ. Atherosclerosis: Recent developments. Cell. 2022;185(10): 1630-45.
48. Yan Y, He F, Li Z, Xu R, Li T, Su J, et al. The important role of apolipoprotein A-II in ezetimibe driven reduction of high cholesterol diet-induced atherosclerosis. Atherosclerosis. 2019;280:99-108.
49. Fan X, Han J, Zhu L, Chen Z, Li J, Gu Y, et al. Protective activities of Dendrobium huoshanense CZ Tang et SJ Cheng polysaccharide against high-cholesterol diet-induced atherosclerosis in zebrafish. Oxidative Medicine and Cellular Longevity. 2020;2020.
50. Liu C, Gates KP, Fang L, Amar MJ, Schneider DA, Geng H, et al. Apoc2 loss-of-function zebrafish mutant as a genetic model of hyperlipidemia. Disease models & mechanisms. 2015;8(8):989-98.
51. Mazzaferro E. Identification and characterization of causal genes for LDL cholesterol levels and downstream effects on atherosclerosis; 2017.
52. Ellman DG, Slaiman IM, Mathiesen SB, Andersen KS, Hofmeister W, Ober EA, Andersen DC. Apex resection in zebrafish (*Danio rerio*) as a model of heart regeneration: A video-assisted guide. International Journal of Molecular Sciences. 2021;22(11):5865.
53. Jopling C, Suné G, Faucherre A, Fabregat C, Izpisua Belmonte JC. Hypoxia induces myocardial regeneration in zebrafish. Circulation. 2012;126(25):3017-27.
54. Cao J, Navis A, Cox BD, Dickson AL, Gemberling M, Karra R, et al. Single epicardial cell transcriptome sequencing identifies Caveolin 1 as an essential factor in zebrafish heart regeneration. Development. 2016;143(2):232-43.
55. Wang J, Karra R, Dickson AL, Poss KD. Fibronectin is deposited by injury-activated epicardial cells and is necessary for zebrafish heart regeneration. Developmental Biology. 2013;382(2):427-35.
56. Ross Stewart KM, Walker SL, Baker AH, Riley PR, Brittan M. Hooked on heart regeneration: The zebrafish guide to recovery. Cardiovascular Research. 2022, May 15;118(7):1667-79.
57. Chablais F, Veit J, Rainer G, Jaźwińska A. The zebrafish heart regenerates after cryoinjury-induced myocardial infarction. BMC Developmental Biology. 2011;11:1-13.
58. Chablais F, Jaźwińska A. Induction of myocardial infarction in adult zebrafish using cryoinjury. JoVE (Journal of Visualized Experiments). 2012;(62):e3666.
59. de Preux Charles A-S, Bise T, Baier F, Marro J, Jaźwińska A. Distinct effects of inflammation on preconditioning and regeneration of the adult zebrafish heart. Open Biology. 2016;6(7):160102.
60. Xu S, Xie F, Tian L, Fallah S, Babaei F, Manno SH, et al. Estrogen accelerates heart regeneration by promoting the inflammatory response in zebrafish. Journal of Endocrinology. 2020;245(1):39-51.
61. Fang Y, Lai KS, She P, Sun J, Tao W, Zhong TP. Tbx20 induction promotes zebrafish heart regeneration by inducing cardiomyocyte dedifferentiation and endocardial expansion. Frontiers in Cell and Developmental Biology. 2020;8: 738.
62. Peng X, Lai KS, She P, Kang J, Wang T, Li G, et al. Induction of Wnt signaling antagonists and p21-activated kinase enhances cardiomyocyte proliferation during zebrafish heart regeneration. Journal of Molecular Cell Biology. 2021; 13(1):41-58.
63. Kikuchi K, Holdway JE, Werdich AA, Anderson RM, Fang Y, Egnaczyk GF, et al. Primary contribution to zebrafish heart regeneration by gata4+ cardiomyocytes. Nature. 2010;464(7288):601-5.
64. Kikuchi K. Advances in understanding the mechanism of zebrafish heart regeneration. Stem cell research. 2014 Nov 1;13(3):542-55.
65. Tahara N, Brush M, Kawakami Y. Cell migration during heart regeneration in zebrafish. Developmental Dynamics. 2016; 245(7):774-87.
66. Marín-Juez R, Marass M, Gauvrit S, Rossi A, Lai S-L, Materna SC, et al. Fast revascularization of the injured area is essential to support zebrafish heart

regeneration. Proceedings of the National Academy of Sciences. 2016;113(40): 11237-42.
67. Ghosh TK, Song FF, Packham EA, Buxton S, Robinson TE, Ronksley J, et al. Physical interaction between TBX5 and MEF2C is required for early heart development. Molecular and Cellular Biology. 2009;29(8):2205-18.
68. Song M. GATA4/5/6 family transcription factors: An ancient guide for cardiac and pharyngeal mesoderm fate decisions: University of Toronto (Canada); 2023.
69. Tahara N, Akiyama R, Wang J, Kawakami H, Bessho Y, Kawakami Y. The FGF-AKT pathway is necessary for cardiomyocyte survival for heart regeneration in zebrafish. Developmental Biology. 2021; 472:30-7.
70. Zuppo D, Tsang M, editors. Zebrafish heart regeneration: Factors that stimulate cardiomyocyte proliferation. Seminars in cell & developmental biology. Elsevier; 2020.
71. Gemberling M, Karra R, Dickson AL, Poss KD. Nrg1 is an injury-induced cardiomyocyte mitogen for the endogenous heart regeneration program in zebrafish. Elife. 2015;4:e05871.
72. Nwaneshiudu A, Kuschal C, Sakamoto FH, Anderson RR, Schwarzenberger K, Young RC. Introduction to confocal microscopy. Journal of Investigative Dermatology. 2012;132(12):1-5.
73. Hove JR, Craig MP. High-speed confocal imaging of zebrafish heart development. Cardiovascular Development: Methods and Protocols. 2012;309-28.
74. Malone MH, Sciaky N, Stalheim L, Hahn KM, Linney E, Johnson GL. Laser-scanning velocimetry: A confocal microscopy method for quantitative measurement of cardiovascular performance in zebrafish embryos and larvae. BMC Biotechnology. 2007;7:1-11.
75. Yalcin HC, Amindari A, Butcher JT, Althani A, Yacoub M. Heart function and hemodynamic analysis for zebrafish embryos. Developmental Dynamics. 2017;246(11):868-80.
76. Benslimane FM, Zakaria ZZ, Shurbaji S, Abdelrasool MKA, Al-Badr MAH, Al Absi ESK, Yalcin HC. Cardiac function and blood flow hemodynamics assessment of zebrafish (*Danio rerio*) using high-speed video microscopy. Micron. 2020;136: 102876.
77. Adams MW, Loftus AF, Dunn SE, Joens MS, Fitzpatrick JA. Light sheet fluorescence microscopy (LSFM). Current Protocols in Cytometry. 2015;71(1):12.37. 1-12.37. 15.
78. Huang Z, Gu P, Kuang D, Mi P, Feng X. Dynamic imaging of zebrafish heart with multi-planar light sheet microscopy. Journal of Biophotonics. 2021;14(5): e202000466.
79. Matovic T. Using zebrafish to characterize cardiomyopathy related mutations in the cardiac actin gene: University of Guelph; 2014.
80. Singh Angom R, Wang Y, Wang E, Dutta SK, Mukhopadhyay D. Conditional, tissue-specific crispr/ cas9 vector system in zebrafish reveals the role of nrp1b in heart regeneration. Arteriosclerosis, Thrombosis, and Vascular Biology. 2023;43(10):1921-34.
81. Sandhu L. Characterization of cardiac actin gene editing in zebrafish using CRISPR-Cas9 technology: University of Guelph; 2019.
82. Sumanas S, Larson JD. Morpholino phosphorodiamidate oligonucleotides in zebrafish: A recipe for functional genomics? Briefings in Functional Genomics. 2002;1(3):239-56.
83. Zakaria ZZ, Eisa-Beygi S, Benslimane FM, Ramchandran R, Yalcin HC. Design and Microinjection of morpholino antisense oligonucleotides and mrna into zebrafish embryos to elucidate specific gene function in heart development. JoVE (Journal of Visualized Experiments). 2022;(186): e63324.
84. Zhao Y, Yun M, Nguyen SA, Tran M, Nguyen TP. *In vivo* surface electrocardiography for adult zebrafish. JoVE (Journal of Visualized Experiments). 2019;(150):e60011.
85. Lin E, Craig C, Lamothe M, Sarunic MV, Beg MF, Tibbits GF. Construction and use of a zebrafish heart voltage and calcium optical mapping system, with integrated electrocardiogram and programmable electrical stimulation. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2015;308(9): R755-R68.
86. Etzion Y, Muslin AJ. The application of phenotypic high-throughput screening techniques to cardiovascular research.

Trends in Cardiovascular Medicine. 2009;19(6):207-12.
87. Gierten J, Pylatiuk C, Hammouda OT, Schock C, Stegmaier J, Wittbrodt J, et al. Automated high-throughput heartbeat quantification in medaka and zebrafish embryos under physiological conditions. Scientific Reports. 2020;10(1):2046.

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